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BIOLOGY OF THE SHAWNEE CAVE SPIDERS.

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General Introduction.

From September 7, 1908, to September 7, 1909, the author held the Speleological Fellowship in Indiana University with residence at the University's Cave Farm three miles east of Mitchell, Ind. The present paper embodies the results of the observations during this time on the habits of spiders in caves and in the laboratory.

The work has been carried on under the direction of Dr. C. H. Eigenmann, professor of zoölogy in Indiana University, to whom I am indebted for suggestions and the loan of his cave literature. I wish to express my thanks to Dr. Charles Zeleny, associate professor of zoölogy, for many helpful suggestions. I am indebted to Dr. Alexander Petrunkevitch for the identification of the specimens; to Dr. A. M. Banta, for the loan of his entire collection of cave spiders, and to Mr. Will Scott, for part of the map of this series of caves. He surveyed and mapped the cave from "1" to "37" or the Upper Dalton (see map, page 321) in the autumn of 1907; the author assisted by Mr. Frank Green surveyed and mapped the Upper Dalton from "37" to "64" in October, 1908.

Previous Work.

Published observations on the habits of cave spiders are limited to a few scattered papers which give lists of species and localities.[1] The best studies published are those of Packard[2] and Banta.[3]

[1]Banks ('06) and Blatchley ('96) give lists of Arachnids from Indiana Caves.
[2]*Mem. Nat. Acad. Sci.*, IV., 1888.
[3]"Fauna of Mayfield's Cave," 1907.

The Species Studied.

Two species of spiders permanently live in the Mitchell caves. One, *Troglohyphantes* (*Willibalda*) *cavernicola* Keyserling, a linyphid[1] is a true cave form; the other, *Meta menardi* Latreille, is an epeiridid and also lives outside of caves. According to Emerton (1902, 190–) the latter arachnid "lives in caves and similar cool and shady places in various parts of this country and also in Europe."

Banta (1907, 65) reports *Erigone infernalis* Key. from the Twin Cave at Mitchell, Ind. I have been unable to find it here, but have taken it in Mayfield's Cave at Bloomington, Ind.

Troglohyphantes has been observed in detail in order to get as far as possible the life history of a typical cave spider. The distribution, food and results of the experiments of *Meta* are given in order to show how an outside form is able to adapt itself to a subterranean life. All notes unless otherwise stated refer to *Troglohyphantes*. The numbers in quotation marks refer to localities in the caves (see maps, pages 321 and 323).

In *Troglohyphantes* there are all degrees of differences in coloration, and in the degeneration of the eyes. The abdomen varies in color from black, dark brown, light to white. The cephalothorax varies from dark, pinkish, light to a white color. The most common combination of colors is a light brown abdomen with a pinkish cephalothorax. The adult females range in length (cephalothorax and abdomen) from 2.4 mm. to 3.7 mm., while the adult males vary from 2.2 mm. to 3 mm.

In the adults the eyes range from eight in number, each with a maximum size of 0.036 mm. in diameter to no external signs of eyes. I have not seen Keyserling's description, but from his figure, which Packard[2] has copied, the eyes are small, and the front middle ones extremely minute.

Physical Environment.

These spiders are found only in total darkness, where the atmosphere is saturated, and in places suitable for the construction of snares. They are never found where the walls are perpendicular with water covering the entire floor; nor are they

[1]Banks, '06, classification.

[2]1888, Plate XV., Fig. 32.

found where the walls and floor are dry. Distance from the entrance does not necessarily limit the distribution if the three necessary conditions are present, nor does scarcity of food limit their distribution to a very great degree.

At "2" a few were collected last fall but none has been found since. This is 200 feet from the entrance in total darkness. At "9" a few more than at "2" have been found; at "10" a great many have been taken and "13" was my best collecting ground. This place is 600 feet from the mouth. On several occasions I have gathered two or three dozen individuals here and such wholesale collecting at one place seemed at the time to exhaust the supply, but a month after such a collection had been made, I have been able to duplicate this record. Many have been collected at "14" and at "19." The latter locality is the "Big Room," 1,700 feet from the entrance. Three were seen at "23" and only a few were observed in a branch at "31." This latter place is in total darkness 200 feet from the mouth of the Lower Twin Cave at "32." Not one has been observed between "33" and "36." A few have been collected in a branch at "38" in the Upper Dalton. This location is in total darkness 150 feet from the entrance. A number were taken from branches at "46" which is 1,024 feet from the entrance. This locality was my second best collecting ground, particularly for the cocoons. A few were taken at "50." One was caught in the "Big Room" at "57" and several snares were seen. At "63" almost a mile from the entrance of Upper Dalton, three were observed and several webs were among the rocks at a "cave-in."

Troglohyphantes is also quite abundant in Hamer's Cave one half mile west of these caves. None was found nearer than 300 feet from the entrance.

This archnid shares as wide a distribution as the blind beetle, *Anophthalmus tenus* Horn (Blatchley, '96). It far outnumbers the latter in individuals, but is less evenly distributed. By actual count there are twice as many females as males in all the collections made from the various caves.

Meta is found both in twilight and total darkness. In the Shawnee Cave they are quite abundant from the entrance at "1" to "4." The latter place is over 200 feet from the entrance

in total darkness. Several were seen at "6," 100 feet from the mouth. An adult male was observed on the roof at "14"; nine days later, it was seen under a rock on the floor and had constructed a snare. One immature specimen was seen in a branch at "23." At "30," 200 feet from the entrance of Lower Twin, a few live, and at "32" and "33" they are quite numerous.

May 17, I collected eighteen *Metas* and placed them on the north wall by a large pile of rocks at "19" in the "Big Room." August 3, I saw six of them. They had scattered along the wall about 50 feet and among the rock pile. Each had built an orb-web and remains of small diptera were seen in the snares. August 17, after a brief search I saw only three of the eighteen. This absence does not mean that they had died, but that it was impossible to find them.

Locomotion.

This arachnid is very agile and is a good runner. Its long legs and slender body enable it to move from place to place, and to avoid an obstacle with much ease. When not irritated it moves along slowly and gently. When stimulated mechanically, it gives a quick jump, runs and dodges whatever obstacle may be in its way. However, it runs only a few inches and if stimulated a second or third time often drops in an instant and hangs to its web. Sometimes it climbs the web to the place from which it dropped, at other times it lets itself down to the floor and then runs off.

Several were placed on the roof among beads of water and other small obstacles. A pencil was used to stimulate and guide them so that they were obliged to run up against the obstacles. They use the first pair of legs as feelers. These are kept well in advance of the other legs and head so that they can detect an object in front of them the length of the body. While walking or running slowly, they are able to dodge an object every time. If caused to run swiftly, they run against the object, or if the object be a low bead of water, they run over it and pass on.

Webs and Snare.

This spider usually spins a web wherever it goes. It is impossible to see a single thread in the cave with a carbide bicycle

lamp unless the thread is coated with water. Quite often on the roof in a slit between two strata of rocks, or in crevices in the walls are found collections of webs which are generally coated with water. These webs do not seem to be of any service to the spider after once spun. They, however, show how it wanders from place to place. It makes a flat sheet-like snare under which it lives. The snare is slightly curved downward and may lie in such a position as to form any angle between 0° and 90° with the floor. The threads are fine and the snare so transparent that it is difficult to see unless it is coated with water. The meshes are so minute that the snare turns water. The snare is supported by many fine threads from the sides, the length of these vary according to the surroundings. When insects fly into the snare they are taken through it by the spider which is on the under side.

Snares are most abundant in the older parts of the caves where the passages are blocked up with clay. At such places the clay banks are more or less perpendicular and the water has washed out many small crevices. Sometimes large blocks of clay tumble down into a heap. Among these heaps of clay blocks and in such crevices as the above the snares are very numerous. In a crevice five inches wide I once saw three snares built one above the other not more than three inches apart. All three were parallel with the floor.

In several places snares are quite conspicuous in the angle between the floor and wall. In such positions the spiders have better routes for travel from one snare to another than in the clay banks.

The third place where snares are found is among the rocks at "cave-ins." The rough cornered rocks appear to form good places for attachment and quite frequently dripping water is present which this species apparently enjoys.

Food.

While in captivity I have fed them small gnats, small flies and the spiderlings of *Meta*.

In the caves the following observations were made: At "13" while watching a male who was trying to court a female I saw

a white-winged dipteron fly into the snare. In an instant the female seized the insect, then ran back to the male. The latter then ran to the other side of the snare. In a few minutes another white-winged dipteron flew into the snare near the male, and he lost no time in seizing it. Several other times these diptera flew against the snare but failed to be entangled in its meshes. In each case the spiders made a dash for the would-be victim while holding one insect in their mandibles. At an earlier date a spider at this same place was seen eating a white-winged dipteron. On various dates at "14" one was caught eating a myriopod; one, a small gnat; and several were caught under an old turtle's shell with thysanurans.[1] At "19" two or three were observed in a mouse trap with some cheese. At "40" one was caught under an old piece of meat with a small white thysanuran in its mandibles. At "43" one was eating a white-winged dipteron, this spider was running on the wall. At "50" one was seen in its snare eating a small myriopod.

This spider is usually very peaceful. Neither in the caves nor in captivity have I ever seen them to show the least signs of pugnacity when they came in contact with each other. Nevertheless it appears certain that they at times eat each other. A few times their remains have been found in the snares. A few remains were observed in the glass cases in which they were sometimes kept in the laboratory. Quite a number of times remains were found in the collecting vials the next morning when three or four were left in the same vial over night.

When bits of dirt were thrown into the snares, the spiders ran away quickly. Blind beetles were caught and tossed into the snares. At the instant when the beetles struck the snare, the spiders ran and with a jump seized the victim. Neither the web, nor spider, nor both together were strong enough to hold the beetle. Large flies were also thrown into the snare with the same results. The spiders always seized their prey and held on tenaciously until the last second. Small flies and mosquitoes were likewise tossed into the snares. In each case the spider made a quick run and with a jump seized the victims and held

[1]Here as elsewhere used in broad sense to include both *Thysanura* and *Collembola*.

on so firmly that both prey and spider were torn loose from the web by picking the prey up with forceps.

Not one specimen has even been seen drinking water. Since they always live in a saturated atmosphere all the water required is probably absorbed through the skin.

In captivity I have fed *Meta* flies, mosquitoes, gnats and various species of other arachnids smaller than themselves. When two or more of them are placed in the same case the largest invariably devours all the others in a short time.

In the caves *Metas* have been observed at various localities eating gnats, mosquitoes, flies and cave crickets. One was seen eating a small moth and another an old dried myriopod.

Enemies.

As far as my observations go, these spiders have no enemies besides themselves. It is very seldom that one can find the remains of a specimen in the webs and as mentioned above no case of fighting has ever been witnessed. At "63" almost a mile from the entrance the only other insect found was the blind beetle. Doubtless carefully repeated observations at this locality will prove that other insects are also present.

Temperature.

During the last three years a series of temperature records[1] have been taken at "19," or in the "Big Room." For 1907 the temperature is as follows: 11.5° C. for January, February, March, April and May; June 11.7°; July 11.9°; August and September 12.7°; October 12.2°; November 11.9°; and December 11.7°. Since food is more abundant and the three necessary physical conditions are evidently suitable at "2," "9" and "38," we can probably attribute the small number of specimens to the uneven temperature. Again, since food is extremely scarce a half mile from the entrance while the number of specimens is few, perhaps the small number of spiders is due to the scarcity of food. Midway between these two localities food is comparatively plentiful, the temperature is practically even throughout the year, and this combination is probably responsible for the great number of individuals.

[1]Eigenmann, '09.

Specimens have been kept in captivity in the laboratory throughout the year. In cold weather they are less active than in warm and are very fretful when the vials become too warm, and often die.

COURTSHIP.[1]

November 16 two were seen copulating. In order to see them I got too close and my breath irritated the web. This caused them to separate. After an absence of five minutes I returned and found them together again. A second time they were disturbed. Returning after an absence of fifteen minutes, they were found close together. While copulating, they were lying one under the other with anterior and posterior ends reversed and with the ventral parts of their cephalothorax in contact. November 19, a pair was seen pairing. December 17, two were observed copulating, these were both under the snare, and the anterior and posterior ends of the cephalothorax were reversed. The dorsal surface of the cephalothorax of the male was pressed against the corresponding ventral part of the female. The male placed his palp on the epigynum once, this lasted only a few seconds, then they parted. December 14 and 22, two couples were seen copulating. Those on the latter date were first seen at 2:25 P.M. and then disturbed by breath. At 2:27 they were together again after the male had circled once around the female. This pair was on top of the horizontal snare all the time. The male used his palps alternatively three times in three minutes, each time lasted only a few seconds. They were disturbed at 2:30. January 4, two were observed copulating. June 7, a male was seen courting a female. Both were under the snare within one and one-half inches of each other. They were first seen at 3:45 P.M. When the male tried to advance toward the female, she caused him to keep his distance, the result of which caused the male to circle completely around her clockwise in six minutes. Most of the time she kept the posterior end of her abdomen toward him, while he had his head facing her all the time. At 4:00 I left them and a half hour later upon my return they were still in the same position. They were placed in a vial, taken to the house and were put in a case. In this

[1]McCook, '93, describes only courtship of outside forms.

case they lived two weeks where they died. This pair like all others when in captivity had no inclination to mate.

Cocoons and Eggs.[1]

One cocoon was made in a glass case October 4. It contained six eggs. Another was made in a case December 11. January 21, one with five eggs was constructed in a vial. Other cocoons were made in vials on the following dates: April 28; May 5, 8, 22, 27 and 31; June 3, 25 and 28; July 8 and 27; August 6, 19 and 21.

On the following dates cocoons with eggs were collected in the caves: October 8, one with two eggs; one with eight eggs, January 20; January 26, one containing eight newly hatched spiders and one with seven eggs; another with seven eggs March 2; March 16, two cocoons, one with eggs and the other with newly hatched; May 24, one with four eggs; July 2, one with four eggs; July 19, at various localities collected seven cocoons, one of which contained eight eggs, and two others each held four young, at various localities in Upper Dalton August 22 collected seven cocoons. Some of these contained young just hatched, and others young ready to leave the cocoons.

The cocoons in the caves are usually constructed in secluded places and are difficult to find unless one examines every little crevice and looks under the ledges of rocks very carefully. Sometimes they are found attached to the underside of rocks lying on the floor, but more often under little ledges of rocks and in the acute angles of small crevices.

In color they are snow white and are disc-like in shape. The flat part of the disc is fastened firmly to the rocks. The average size is 6 mm. in diameter by 3 mm. in depth, although sometimes a cocoon containing the minimum number of eggs is as large as one containing the maximum number. I have never been fortunate enough to witness a female making her cocoon, but on examination, a coocon is composed of a more or less firm and closely woven circular base. The eggs are piled into a heap in the middle of this base and then the convex part is spun over them in such a crude and unsubstantial way that one can generally count the eggs through this covering. In detaching the

[1]Montgomery, '06, describes the cocoons and eggs of an allied outside form.

cocoon from the rock one must use precaution for fear the eggs fall through the covering.

In number the eggs vary from two to eight with five for an average cocoon. They are transparent whitish in color and are perfect spheres with an average diameter of 0.6 mm. During the embryological stages, they soon take on a yellowish color, become oblong in shape, and the outline of the embryos is discernible through the covering. Some of these embryos assume the shape of the profile of a man's head.

YOUNG.

When hatched they remain for an indefinite period inside the cocoon and when strong enough emerge through a small circular hole.

March 22, three of the seven eggs in a cocoon collected March 2, hatched; April 3, two of these spiderlings were dead, they with the remaining eggs were covered with mold. June 4, two of the four eggs in a cocoon collected May 24, were hatched, one spiderling was dead and the other alive on this date. Neither one had any eyes. July 29, a cocoon collected July 19, was examined and contained three young. Each one was examined both alive and dead. All eyes, except the anterior middle ones, were discernible. Female no. 139 made a cocoon and laid four eggs May 5. On May 23 all four eggs were hatched, but the young were still inside the cocoon. Each spiderling had all eight eyes except the anterior middle ones. The eyes had a uniform diameter of 0.018 mm. While alive under the microscope their little eyes shone like small electric lights. Their mother had no signs of external eyes. Many other newly hatched spiderlings were observed both alive and dead. The anterior middle eyes are never discernible. In some, the other six eyes are present and in others no eyes can be seen. All the other eggs laid in the laboratory failed to hatch. Perhaps this was due to uneven temperature.

The young are much thicker-set than the old. The legs are thick and stubby. The cephalothorax and legs are transparent whitish while the abdomen is light cream in color. The latter has a few longitudinal rows of hairs. The length varies from 0.6 to 0.8 mm.

Moulting.

While in captivity seven individuals moulted, three of which were found dead shortly after the skins were cast off. The deaths were probably partially due to an excess of water in the vials for immediately after the old skins were shed the spiders lay lifeless in the water. The skins were suspended by threads to the upper side of the vials. The moults show that the skin splits on each side of the cephalothorax at the dorsal side of where the legs are attached. Hence, the moulted skin of legs and mouth parts adhere to the ventral half of the moult, and the corneal cuticle belongs to the dorsal half. All the old hairs are shed along with the skin, new ones take the place of the old which causes the color to be perceptibly brighter. The moulted skins of the abdomen were either missing or rolled up into little wads so that one could not tell precisely how they were cast off.

Mortality.

In the caves one never finds dead specimens. In captivity mortality is not great. The most important requirement is to place them in a saturated atmosphere as soon as caught. Two thirds of the specimens were found dead in the collecting vials the following morning when left in the vials without a drop of water over night. It is impossible to keep them long in anything not air-tight, however careful one is to keep them supplied with water. The best device is small vials with air-tight cork stoppers. In such vials they may be kept for months without food. One caught September 10 was placed in a small vial containing two drops of water. On January 20 more water was added which almost drowned the specimen. On January 26 it died. During all this time it had had nothing to eat. Another individual was placed in a small vial January 7, and died April 19, due to lack of moisture or food.

In captivity they quite often die soon after moulting. In vials sunshine kills them in a few minutes. The heat from a student's lamp is also fatal.

Light Experiments.

In the caves one may throw the light from a carbide bicycle lamp on *Troglohyphantes* for a half hour or more without pro-

ducing any effect. Such is not true with *Meta*. Just as soon as the light strikes their eyes, they run into the dark. If the light is repeatedly thrown on their eyes, they may be turned in any direction and often can be driven into places where the light cannot reach them.

The following apparatus was used in the laboratory: For the adult *Metas*, slender 10-inch bottles; for the medium sized *Metas*, 6-inch test-tubes; for the spiderlings of *Meta* and for *Troglohyphantes*, small 5-inch vials. The closed end and the lower half of each vessel were covered with black carbon paper. The open ends were securely closed with air-tight cork stoppers. One specimen with one or two drops of water were placed in each vessel. In a very short time the water forms a thin film of moisture all over the inner surface of the vessel. This saturated the air in both ends equally. The vessels were then placed on an inclined rack by a south window in order to give each an equal amount of light. Occasionally they were rotated so that the light always fell directly upon the spiders' eyes. At various times the carbon paper was transferred to the cork end, thus throwing the specimen into the light or dark as the case may be. Those that were strongly negatively phototropic never lost much time in finding the dark end, regardless of the number of times the carbon paper was changed. Such individuals often pass into the dark in three minutes. The few that were strongly positively phototropic always changed from the dark end to the light end whenever they were thrown into the dark. Some were thus experimented with for thirty days, but experience taught that their actions were reliable for only the first four or five days. Darkness and cloudy weather had much to do with the final results. Time was counted from the period when first placed in the vessel, and each morning when first observed until 6:00 P.M. each day for four consecutive days. When first placed in the vessels and for a short time after the carbon paper was transferred, they were noticed every few minutes, after that irregularly five times every day.

The adult *Metas* were always in the dark end during clear and cloudy weather, and always in the light end when it was dark. The medium sized *Metas* were always found in the dark

end in clear weather, one half the time in the dark end during cloudy weather, and usually in the light end when dark. The spiderlings of *Meta* remained in the dark end one half the time in clear weather, one third the time in the dark end during cloudy weather and most of the time in the light end when dark.

All eight eyes in the *Metas* were present and presumably well developed. The anterior middle ones were generally a little smaller than the others and occasionally an eye was found among the others which was about one half size. In all these experiments forty *Metas* were used.

The following table gives the results of the light experiments for *Troglohyphantes*. On the left is entered the number of the specimen, the locality, the sex, age (whether mature or immature) and the four sets of eyes, AME stands for anterior middle eyes, PME for posterior middle eyes, ASE for anterior side eyes, and PSE for posterior side eyes. All measurements were made with a micrometer slide inside the two inch ocular with two thirds or low objective. When the eyes were scarcely discernible the one-inch ocular was substituted for the two-inch. As a unit of measurement for the eyes one fifty-fifth of a millimeter (0.018 mm.) is employed. The fractional parts of this unit are only approximate. The +'s are used when the eyes are joined together, if separate −'s are employed. P stands for pigment speck. The remainder is self-explanatory.

The thirty examples included in the table were selected, not on a phototropic basis, but to represent the various localities, the age, size, degeneration of the eyes, and coloration. If another table were made from the specimens not included in this one, the results would be similar. If a correction could be made for cloudy weather and for the time occupied in going into the dark, the total per cent. of 49 for those in the dark column would be considerably larger than the total per cent. of 51 for those in the light column. In all these experiments 225 specimens have been used and I am positively certain that the results as given in the table are correct.

Summarizing the following table and other data not included therein we have the following statements:

Twenty-six per cent. of all the individuals examined had no

LIGHT EXPERIMENTS FOR TROGLOHYPHANTES CAVERNICOLA KEY.

No.	Loc.	Sex.	Age.	Size.	AME.	PME.	ASE.	PSE.	Color Abd.	Color Ceph.	Light. Hrs. and Min.	Dark. Hrs. and Min.	Light. Per Cent.	Dark. Per Cent.
397	9	♂	Im.	2.2	.5+.5	.7—.7	.7— .7	.7— .7	light	pink	11.40	24.45	38	62
712	9	♀	Mat.	2.8	.5—.5	1—.5	1— 1	1.5—1.5	brown	"	15.20	21.05	42	58
322	10	?	Im.	1.0	0— 0	0— 0	0— 0	0— 0	light	light	10.00	25.15	28	72
324	10	♂	"	2.5	.5+.5	1— 1	0— 0	0— 0	"	pink	15.15	20.00	43	57
327	10	♀	Mat.	2.6	0— 0	0— 0	0— 0	0— 0	brown	"	13.00	22.15	37	63
714	10	♀	Im.	2.7	.5+.5	1— 1	1— 1	1— 1	"	light	8.25	28.00	23	77
325	13	♀	Mat.	2.8	1— 1	2— 2	1.5—1.5	1— 1	"	pink	30.15	5.00	85	15
326	13	♀	"	2.5	1+ 1	2— 2	1— 1	1— 1	light	"	18.00	17.15	51	49
399	13	♀	"	2.8	.5+.5	.5—.7	1— 1	1— 1	"	"	17.55	18.30	45	55
705	14	♂	"	2.7	.7+.7	1— 1	1— 1	1— 1	"	"	23.10	13.15	63	37
747	14	♀	Im.	2.0	0— 0	2— 2	2— 2	2— 2	"	"	2.45	33.35	8	92
729	14	♂	Mat.	2.8	.7—.7	1— 1	1— 1	1.5—1.5	"	"	22.05	14.15	60	40
741	14	♀	"	3.0	0— 0	0— 1	.7— 1	0— 1	"	"	15.50	20.30	44	56
709	19	♀	"	3.0	0— 0	0— 0	0— 0	0— 0	"	"	16.05	20.20	45	55
731	19	♀	"	3.0	.7— 1	1— 0	1— 1	1.5—1.5	brown	"	30.30	5.50	83	17
735	19	♀	"	3.1	1— 1	1— 1	0— 1	0—1.5	dark	"	30.50	2.30	91	9
736	19	♀	"	3.0	.7— 1	1— 1	0— 1	1.5— 2	light	"	36.20	0.00	100	00
359	38	♀	"	2.8	.5—.5	P— 1	0— 0	0— 1	brown	"	13.05	21.40	38	62
374	38	♀	"	2.9	.5—.5	.7—.7	.7— 0	.7— .7	"	"	29.30	9.00	76	24
356	38	♀	?	?	.5—.5	0—.7	0— 0	0— 0	?	"	28.05	6.40	81	19
355	40	♀	Mat.	2.8	.5— 1	.5—.5	1— 1	1— 0	brown	"	15.10	19.35	43	57
371	40	♀	"	3.0	0— 0	0— 0	0— 0	0— 0	light	"	2.25	32.20	7	93
375	43	♀	Im.	3.0	0— 0	0— 0	0— 0	0— 0	dark	"	16.30	22.00	43	57
753	43	♀	Mat.	3.2	.5— 1	1— 1	.7— .7	0— 0	"	"	33.40	0.00	100	00
755	43	♀	"	2.4	1— 1	1— 1	0— 0	1— 1	"	light	25.45	7.55	76	24
725	46	♀	"	3.0	.7—.7	1— 1	.7— 0	1— 1	brown	pink	12.40	23.00	35	65
726	46	♀	"	2.9	0— 0	1— 1	1— 0	1— 1	black	"	10.25	25.15	29	71
721	50	♀	"	3.2	.7—.7	1— 1	1— 1	1— 1	dark	"	14.55	21.20	41	59
722	50	♂	Im.	2.3	0— 0	0— 0	0— 0	0— 0	light	light	12.05	24.10	33	67
331	57	♂	"	2.0	0— 0	0— 1	0— 0	0— 0	black	pink	17.45	17.15	51	49
										Total	549.25	522.30	51	49

[? Abdomen was lost.]

external eyes. Sometimes the eyes are not in their natural position. Often black pigment specks are found where the eyes are absent. The largest eyes are two fifty-fifths millimeter (0.036 mm.) in diameter, being twice as large as those of the newly hatched, but such individuals are comparatively rare. Hence as a rule, the eyes do not grow larger after birth, while the specimens more than thribble themselves in size. Neither locality nor size of the specimen determines the degree of degeneration in the eyes, or the shade of coloration. Generally, the lighter colored the individual, the more degenerated the eyes, and vice versa. Specimens totally devoid of eyes always stay in the dark more than fifty per cent. of the time; those with one or more

eyes may stay either in the light or dark more than fifty per cent. of the time, the per cent. depending on the amount of degeneration.

Humidity.

Apparatus.—Glass tubes with one-half-inch bore and twelve inches long were used. The opening and three inches of one end were covered with black carbon paper. The other end was closed with a cotton cloth. A spider and two drops of water were placed in the light end of each tube and the tubes were placed in the light the same as in the light experiments. When first placed in the tubes the specimens wandered from one end to the other. In just a few moments they ceased their wandering and remained within reach of the drops of water. As it was impossible to watch these experiments all the time, quite frequently the drops of water evaporated before new ones could be added. Sometimes when the tubes became dry, the spiders were found in the dark end, other times in the light end. Under such conditions some were able to live only one or two days, some four or five days while others survived ten days. Out of two dozen individuals used not one at any time was ever found in the dark end when the light end was wet. Each specimen was examined, some had eyes and others were devoid of eyes. Judging from the preceding experiments on light the specimens devoid of eyes should have been found in the dark end at times, providing there was no other factor stronger than negative phototropism. Since these specimens remained near the drops of water all the time instead of going into the dark, we conclude that humidity is a stronger factor than negative phototropism.

The same experiments were prosecuted with the spiderlings and medium sized specimens of *Meta*. At times these were found in the dark end when the light end was wet, therefore probably humidity with them is not greater than negative phototropism.

Change of Humidity.

Apparatus.—The same tubes as used in the preceding experiments for humidity, also a hygrometer was employed. A specimen was placed in each tube and was observed several times each day. The following results show the relative humidity and the number of hours and minutes various individuals lived.

No. of Specimens.	Relative Humidity.	Hrs.	Min.
4	38—36	3	15
2	70—66—59	5	30
1	38—36—44	8	15
4	63—93	8	15
1	95—82—84—88— 92—78	23	35
1	81—60—76—73—100—75—89	31	45
1	100—65—55—60— 72—66—60	33	45

On various dates at the entrance and at the different localities in the caves the relative humidity was recorded. At the entrance it varied considerably on different days, but in the caves, the hygrometer always stood at 100 (saturation point).

These arachnids always live in a saturated atmosphere and it is impossible for them to survive long outside the caves where the variation in the degree of humidity is great. As a general rule the higher the relative humidity (with but a gradual and small amount of variability), the longer they live. Since the above experiments were prosecuted from May 18 to June 8, when the change in temperature was not such as to materially affect these spiders, we must attribute their deaths to the hygrometric conditions.

Summary.

1. *Troglohyphantes cavernicola* Keys. is found everywhere in these caves, where the three following necessary conditions exist—total darkness, a saturated atmosphere, and a suitable place for the construction of snares.

2. The first pair of legs are used as tactile organs.

3. All small winged insects, thysanurans and small myriopods serve as food. Scarcity of food does not entirely limit their distribution.

4. They have no known enemies other than themselves.

5. While temperature outside the caves does not materially affect the adult spiders themselves, it is probable that to the even temperature at localities between 600 and 1,700 feet from the entrance is due the great number of specimens found at this place.

6. Courtship is similar to that of some outside forms.

7. Cocooning is rudimentary. The eggs are few and comparatively large.

8. The young are white and are thicker set than the adults. Some are hatched with eyes, while others are entirely blind.

9. Moulting is comparatively rare and is often fatal. There are all shades from white to black in coloration.

10. In size the eyes vary from a small pigment speck to 0.036 millimeter in diameter. As a rule, after birth the eyes cease to grow while the specimens more than thribble themselves in size. Twenty-six per cent. of all the individuals are entirely devoid of eyes.

11. The degree of degeneration in the eyes and the shade of coloration are not determined by either locality or size of the specimen.

12. The lighter colored the specimen the more degenerated the eyes.

13. The more degenerated the eyes the greater the negative phototropism, and vice versa.

14. Humidity is a stronger factor than negative photropism in determining the location of specimens in the experimental tubes.

15. Change of relative humidity is fatal in a few hours.

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EXPLANATION OF MAP.

Shawnee Cave (the outlet). Sec. I., No. 1.
Closed chamber caused by collapse of roof at Sec. I., Nos. 2–3.
Cascade. Sec. I., No. 6.
Double passage. Sec. I., Nos. 7–8.
Old cross cave. Sec. I., Nos. 9–10.
New passages. Sec. I., Nos. 1–8 and 11–13.
Opening in roof leading to *upper older* levels of cave. Sec. I., No. 14.
"Big Room." Sec. I., Nos. 15, 16, 17, 18, 19, 20, 21, 22.
"Fallen Rock." Sec. I., No. 31.
Lower Twin Cave. Sec. I., No. 32.
Upper Twin Cave. Sec. I., No. 33.
Roof too low for passage of boat. Sec. I., No. 34.
Deepest water in cave, 10 feet 4 inches. Sec. I., No. 35.
Lower Dalton Cave. Sec. I., No. 36.
Upper Dalton Cave. Sec. I., No. 37.

FIG. 1. Map of Shawnee Cave, section 1, from Shawnee to Lower Dalton. Length 4,453 feet. Scale 200 feet to the inch.

EXPLANATION OF MAP.

Upper Dalton Cave. Sec. II., No. 37.
"Cross bedding" in limestone. Sec. II., Nos. 46–47.
"Old passages." Sec. II., Nos. 56–57.
Obstruction past which boat cannot be taken. Sec. II., No. 63.
End of exploration. Sec. II., No. 64.

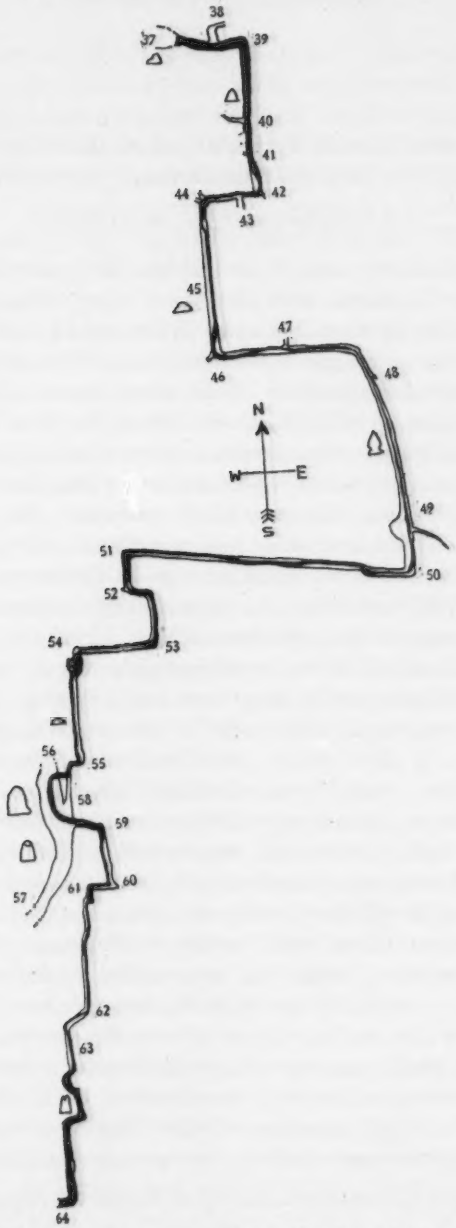


FIG. 2. Map of Shawnee Cave, section 2, from Lower Dalton to unexplored part. Length 4,674 feet. Scale 200 feet to the inch.

A NEW SPECIES OF PARAMECIUM (P. MULTIMICRONUCLEATA) EXPERIMENTALLY DETERMINED.[1]

J. H. POWERS AND CLAUDE MITCHELL.

On September 27, 1909, I received from Dr. Powers two sample cultures of Paramecia with the request that I investigate them as to type and purity of culture. To this end I first killed, fixed, mounted and examined 1,000 individuals. They proved to be neither typical *Paramecium caudatum* nor *Paramecium aurelia*, although most of their characters differed but little from these well-known types. Their length ranged between 144 and 288 μ. Their anterior end was a little blunter and the posterior end a little more pointed than even in *P. caudatum*. The cytoplasm was more dense and more opaque. Their chief difference, however, from hitherto described types of Paramecia lay in the matter of the micronucleus, for, instead of the single micronucleus of *P. caudatum* or the two micronuclei of its variety *P. aurelia*, there is a number of very small bodies, evidently micronuclei, ranging in diameter from about .7 to 1.15 μ (Fig. 3). The characteristic position of micronuclei is fully retained, these bodies lying either in slight grooves or in shallow impocketings of the macronucleus. Like the micronuclei of other types, these delicate bodies are always surrounded by a nuclear membrane.

Of the 1,000 individuals examined 875 distinctly showed from two to six of these small micronuclei, 124 showed apparently no micronuclei whatever, while one appeared at first to possess a micronucleus of the type found in *P. caudatum*. This single instance, however, turned out upon careful study to be a case in which a detached fragment of the macronucleus chanced to simulate in size and appearance the regular micronucleus. As to the 124 which appeared without micronuclei, entire degeneration of these bodies may have been possible, but it is more probable that a slight overstain obscured them, especially when lying behind the macronucleus; the same explanation is doubt-

[1] Studies from the Zoological Laboratory, the University of Nebraska, No. 101.

less true of the individuals in which but two or three micronuclei were found, others existing but in a less visible location.

Finding thus that the cultures in hand contained nothing but this same type of *Paramecium*, I next proceeded to test the permanence of the type. On October 9, I isolated five individuals, placing each in a clean watch glass containing a definite proportion of sterile and bacterially infected water. These individuals, however, lived but a day. I then again selected five more, varying the proportions of the fluid media. Of these, the two which were placed in water containing the highest percentage of bacteria lived, while the others did not. Three more were started in the same manner as the two successful ones and all these gave living cultures. Of the five living cultures thus obtained two proved much stronger than the others, despite the fact that the conditions were kept as constant as possible in all cases; these two increased rapidly in numbers, while the others increased but little and finally died out after five weeks.

From these two strong cultures, forty individuals were killed, stained and mounted on November 22 and about fifty more on December 17. All of these proved identical in type with the original wild stock. The minute micronuclei were present as before, and again seemed to vary from three to seven in number, which difference depended, in part at least, upon the stain and the transparency of the individual.

Unfortunately during Christmas week extreme cold weather and partial failure of heating plant caused the death of all isolation cultures, the original culture, however, remaining. From this latter, single individuals were again isolated and new cultures started on January 31, the culture media being varied as in previous instances. This resulted again in cultures of varying degrees of strength. One of the best, which I will designate as culture X, was chosen and tested by mounting a number of individuals all of which again proved to be of the multimicronucleate type. This culture X was now accordingly taken as a basis for all further work. From it six cultures were started, the medium being modified in this case by the use of different proportions of agar agar infected with the customary bacteria.

Of these six cultures one, culture Y, was worthy of especial

note in that it produced a few conjugants. Early in April this culture became infected with a minute unicellular alga and, possibly as the result of this, the paramecia became more active and increased more rapidly in number. They also ingested the algæ until they became greenish in color. On April 15 six pairs of conjugants appeared. Three of these were killed in about the three-hour stage of conjugation, another in about the seventeenth hour of conjugation, while the other two pairs were isolated, allowed to complete the act of conjugation, and the ex-conjugants used to start new cultures. It was hoped that stronger cultures would thereby be obtained, but this did not follow. They lived and divided slowly for about three weeks only.

The pairs of conjugants which had been killed were stained and mounted in toto, and are of interest as showing, not only that this type of *Paramecium* is capable of conjugation, but something of the nuclear phenomena undergone during the process. In all cases the micronuclei, or at least a part of them, could be made out. In those killed at the three-hour stage (Fig. 1) all were in pairs, indicating no doubt the customary divisions preceding nuclear exchange. In one case three of these pairs were really single nuclei in advanced division. With difficulty the nuclear membrane could be made out, extending, as in the case of the larger dividing micronuclei of *P. caudatum*, between the separating portions of the dividing nucleus. The micronuclei forming the pairs in these three-hour conjugants were smaller than those in non-conjugants. The macronucleus in this stage is still unchanged except that its surface is more or less furrowed.

In the pair of conjugants killed at the seventeen-hour stage the micronuclei are also present, some again in pairs or in division, some single. The macronucleus on the other hand has now broken up into bands and curved segments, simulating a reticulum. This breaking up of the macronucleus at an early stage does not occur with *P. caudatum*, and, in case further study shows it to be habitual with the present type, this will constitute further proof of its independence.

The limited number of conjugants at our disposal and the consequent inability to procure all the stages have prevented

our demonstration of the actual nuclear exchange during conjugation, but such exchange is naturally to be inferred from the preparatory division of the micronuclei and from subsequent breaking down of the macronucleus. Every feature of the few conjugating pairs examined indicated normal processes and conditions.

Although the cultures started from the ex-conjugants fared so poorly it is worth noting that shortly after the latter were discovered in culture Y this culture underwent a rapid acceleration in growth and division. This may have been due to undetected cases of conjugation occurring in the culture, or on the other hand it may have been the result of some external stimulus, which itself had caused the conjugation. From this rapidly growing culture two further lots of Paramecia have been mounted, and, as before, all prove to be of the multimacronucleate type. Sections have also been made and stained with iron hæmatoxylin (Fig. 3) from different groups mounted during the year. They have fully borne out the results of the more numerous toto mounts.

All told the cultures have been conducted, the first group for three, and the second for five months. They have not been as strong as could be wished, but the best cultures obtained showed at least no tendency to die out and at the close of the work culture Y was multiplying more rapidly than at any previous time. Had our temperature conditions been more uniform and favorable, we should probably have been able to rear much more copious cultures. The entire uniformity of the type throughout these cultures seems good evidence for its permanence and the probability that it deserves specific rank.

CLAUDE WM. MITCHELL.

I can fully vouch for the methods and results which my student, Mr. Mitchell, has recorded in the first part of this paper. I may speak a few words further as to my own experience with *P. multimicronucleata*. It is not, in the writer's vicinity, a rare or accidental type. Throughout a number of years of work in eastern Nebraska it has been a frequent and troublesome intruder in my *Paramecium* cultures. The most persistent efforts

have often failed to procure, from wild stock, pure cultures of *P. caudatum*. A portion, usually the bulk, and frequently the whole, of any culture obtained from pond or river water would turn out to be of this multimicronucleate type.

I did not at first recognize the minute micronuclei. I regarded the individuals, which careful and elaborate technique showed to be lacking in the typical micronuclei of *P. caudatum* and *P. aurelia*, as degenerates in the sense of Maupas' contention. As however the hypothesis of the degeneration of the micronucleus became more and more discredited, I reëxamined mounted slides of these Paramecia under high magnifications, with the result that the minute bodies in question were visible in every case. That this type of *Paramecium* was not related to degeneration was further shown by the fact that many pure cultures, unlike those with which Mr. Mitchell has labored so assiduously, have been vigorous and strong growers. I may further mention the fact that in several very large aquaria supplied with running water and a small amount of fresh meat added occasionally, this type of *Paramecium* apparently maintained itself continuously for several years. As often as the organic matter was supplied the animals would multiply and appear in vast swarms in the corners and protected portions of their space; whenever examined they proved of this type and of this type only.

The existence of an undescribed species of *Paramecium* seems improbable. The protozoa are considered of universal distribution, and *Paramecium* is the most-studied genus in existence. Nevertheless much of the study of mircoörganisms is superficial; many have failed to develop a suitable technique, easy as this is, for the certain demonstration of micronuclei; and as to the hypothesis of universal distribution, it is certainly assumed much further than it is proven. Thus, for the last six years, I have made careful search among cultures derived from very numerous wild stocks, for *Paramecium* of the *aurelia* type, *i. e.*, with the well-known two micronuclei. But, aside from a very few isolated individuals derived experimentally from *P. caudatum*, not a single example has been found.

All in all, it seems that, in the light of Mr. Mitchell's results, the type in question deserves specific rank, although this rank

rests upon nuclear differences only. The external characters mentioned by Mr. Mitchell, although holding good for the material investigated by him and for many other lots as well, are not universal, nor have I noted any external character that is. When *P. multimicronucleata* is grown in the same culture with any given strain of *P. caudatum*, the two can usually be readily separated by one or more external characters. Frequently the *P. multimicronucleata* are uniformly larger than the accompanying *P. caudatum*; but they do not exceed the known dimensions of the commoner form, and, in some cultures they are uniformly smaller. So with length of groove, form of ends, opaqueness, etc. The most uniform character that I have seen is that the new type is a little more cylindric in form, bulging less at the point of greatest diameter; but *P. caudatum* approaches this form in starved cultures.

The propriety of basing a species upon nuclear characters only depends of course upon their constancy. Calkins has shown that the types of *Paramecium* with single and double micronuclei respectively are not wholly constant, occasional transitions taking place in both directions. He, therefore, pronounced the types varieties. But the conclusion has again been called in question, the infrequency of the transition leading Kofoid to re-interpret the phenomena as instances of mutation.

I have myself been conducting, during a considerable part of the present year, preliminary experiments on *P. caudatum*, subjecting them to different conditions with a view to ascertaining their possibilities of variation. The only striking results have occurred as the consequences of great changes in feeding habit.

P. caudatum is almost exclusively a bacteria feeder. But as Mr. Mitchell has recorded they occasionally deviate to other minute vegetable organisms. This year I have succeeded in inducing a certain percentage of the individuals from a pure culture of very large and strong growing *P. caudatum* to feed on minor animal organisms, first on flagellates (*Chilomonas*) and then, to a considerable extent, upon smaller ciliates. These very striking changes in food habit produced very striking variations in the Paramecia, both nuclear and cytoplasmic. I will not

describe these at the present time save in so far as they relate to the present discussion. Many of the nuclear changes were erratic and possibly pathological: Macronuclei greatly enlarged, micronuclei unchanged, or sometimes apparently absent, or again enlarged, even more in proportion than the macronucleus, and sometimes divided.

Among the large mass of such material, stained, mounted and examined, I discovered a very few instances of individuals with two typical micronuclei. In fission these micronuclei divided simultaneously and normally. The number of these individuals was very few, probably not exceeding one to several thousand, but they confirm, to some extent, Calkins's observation that *P. aurelia* may arise from *P. caudatum*.

Among the different types of variants I sought assiduously for examples of *P. multimicronucleata*. But none of the exact type were found. Evidently this type is farther separated from *P. caudatum* than is *P. aurelia*. A considerable number of individuals were found however which showed an approach to *P. multimicronucleata*, in that the micronucleus was divided, usually, again, into but two bodies, perfectly normal in appearance, but much smaller than the typical micronuclei of the genus, though a little larger than those of the new type. This variant was one of the most constant and frequent results of the changed diet. In other characters, however, it did resemble closely *P. multimicronucleata* or, for that matter, any recognized type of the genus. I regard it merely as an instance of the well-known law that a powerful stimulus to variation applied to any species brings out, not only new characters, but characters of existing allied species as well. The phenomena, to the writer, serve to confirm, rather than to refute, the specific independence of the new type. But they are of interest in themselves as showing possible lines of experiment leading to nuclear variation. In the present instance it seemed especially worth while to record them, and indeed this is the chief reason for the entire study, in that *Paramecium* is more and more being made the subject of extensive experimental research. So far, little of this study has had regard to other than external characters, but this admitted limitation must soon be remedied, and to this end it is essential that we know the types

of nuclear structure present in the different species or varieties of the genus as well as the lines of variation to which they are subject.

J. H. POWERS.

UNIVERSITY OF NEBRASKA,
July 30, 1910.

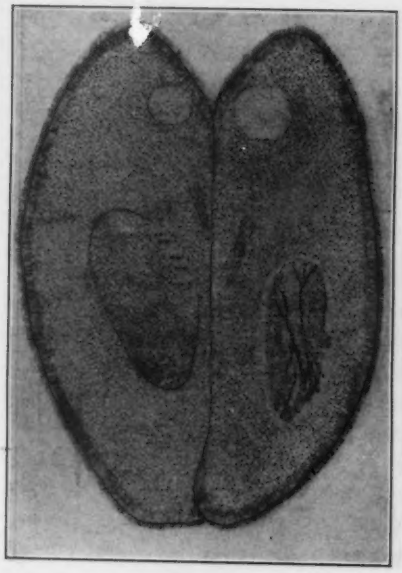
EXPLANATION OF PLATE I.

Paramecium multimicronucleata.

FIG. 1. Conjugation near the three-hour stage, showing micronuclei in pairs or in division.

FIG. 2. Conjugation at about seventeen hours. Macronucleus already broken into band-like portions. Micronuclei visible in part, in pairs or single.

FIG. 3. Section of typical *P. multimicronucleata*, an unusual number of the micronuclei chancing to lie in one plane.



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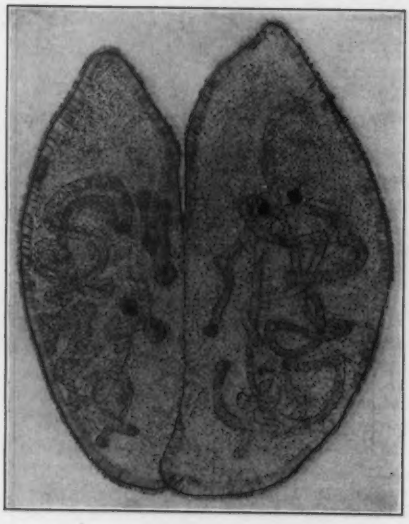


2

THE CENTRAL NERVOUS SYSTEM AS A FACTOR IN THE REGENERATION OF POLYCLAD TURBELLARIA.

C. M. CHILD.

Some years ago L. V. Morgan[1] described a series of experiments concerning the effect of removal of the cephalic ganglia upon the course of regeneration, particularly of the anterior region, in the Californian polyclad, *Leptoplana littoralis*. Her conclusion is that under certain conditions, *i. e.*, particularly in the presence of tissue anterior to the ganglia, regeneration occurs as readily and as completely in the absence of the ganglia as when they are present. At about the same time the results of my own experiments on *Leptoplana tremellaris* appeared.[2] I found that when half or less than half of the ganglionic tissue was removed, regeneration might be as complete and as rapid as when the ganglia were uninjured, but that when more than half of the ganglion was removed, regeneration was not only slower but less complete than when they were uninjured.

I also found, however, that groups of eye spots not infrequently appeared in cases where the ganglia themselves were, so far as could be determined, completely removed, and that the amount of anterior new tissue in all such cases was greater than in those cases where eye spots did not appear, though regeneration was never complete.[3] My general conclusion from these experiments was that the central nervous system in these forms acted as a functional stimulus to the growth of new tissue, increasing both the rapidity and the amount of growth.

The above mentioned experiments of L. V. Morgan seem to

[1]L. V. Morgan, "Incomplete Anterior Regeneration in the Absence of the Brain in *Leptoplana littoralis*," BIOL. BULL., IX., 3, 1905.

[2]Child, "Studies on Regulation, "V.—The Relation between the Central Nervous System and Regeneration in *Leptoplana*: Posterior Regeneration," *Journ. Exp. Zoöl.*, I., 3, 1904. "Studies on Regulation, VI.—The Relation, etc.: Anterior and Lateral Regeneration," *Journ. Exp. Zoöl.*, I., 4, 1904.

[3]Child, "Studies on Regulation, VI.," Figs. 15–19, p. 522, Figs. 41–43, p. 526, also p. 529.

indicate that under certain conditions this is not the case. In view of the apparent disagreement between her results and my own further experiment seemed desirable and during the autumn of 1907 and the summer of 1910 I took the opportunity to examine several species of Leptoplanidæ which occur at La Jolla, Calif., with reference to this point. In 1905 I had worked with *L. littoralis* at Pacific Grove and obtained results similar to those described for *L. tremellaris*.

My conclusions from this later work are essentially the same as those reached in my earlier paper. Removal of the ganglia with as little of the surrounding tissue as possible always results in decreased rapidity and completeness of regeneration, whatever the method of operation employed. In many cases, however, groups of eye spots appear in the new tissue, even when the ganglia are wholly absent, and in such cases the regeneration is always more rapid and more nearly complete than when the eye spots do not appear.

When the ganglia are removed by a cut from one side of the head, as in some of Morgan's experiments, more new tissue is often formed, or it forms more rapidly, in the deep cleft made by the cuts than on a nearly flat terminal surface. This, however, is not due to any specific effect of the anterior tissue, but is merely a very general characteristic of wound-healing, not only in *Turbellaria*, but in many other forms, and is doubtless due to the fact that nutritive and other conditions for growth are better in such a cleft, where the growing parts are in contact on both sides with other tissue, than on surfaces where such contact exists only on one side.

I believe that the important point in connection with the problem of the influence of the central nervous system on regeneration in these forms lies in the question as to what constitutes the central nervous system. As Morgan states, the cephalic ganglia in the polyclads are enclosed in a definite sheath, but a further point of great importance which she does not consider at all is that the nerve roots contain numerous ganglion cells for a considerable distance from their point of origin in the ganglia. Reference to Lang's monograph of the polyclads[1] is sufficient to

[1]Lang, "Die Polycladen des Golfes von Neapel," *Fauna und Flora des Golfes von Neapel*, XI., Leipzig, 1884.

establish this point. There is then every reason to believe that the central nervous system comprises, not the ganglia alone, but the ganglia plus the nerve roots for a certain greater or less distance from their origin. Even when the ganglia are completely removed, the capacities of the central nervous system for regeneration and stimulation are not wholly lost, if sufficient portions of the nerve roots near the ganglia remain. In such cases the amount of regeneration is greater than when the nerve roots are also removed, and groups of eye spots may appear. In fact, in one case[1] I observed the regeneration of a small but distinct ganglionic mass after the apparently complete removal of the ganglia. It seems not improbable that if our technique were sufficiently exact to permit removal of the ganglia without injury to the nerve roots except at their origin, the regeneration even of the ganglia themselves, as well as of other parts, might be almost or quite as complete as when the ganglia remain.

L. V. Morgan's Fig. B2 of the regenerated anterior end of the nervous system after removal of the ganglia shows only fibrillar structure and she states that only fibrillæ are present in the mass. But when we recall the facts as to the histological structure of the nerve roots it seems extremely improbable that ganglion cells are totally absent from such regenerated masses. In all cases of the kind, which I have observed, some cells as well as the fibrillæ have always been present in the knot of tissue formed by the union of the nerve roots.

The development of eye spots in many of the cases without ganglia described by Morgan is undoubtedly due to regenerative processes in the remaining ganglionic nerve roots. In my own experiments I have found that in all cases, whatever the method of operation, where the ganglia plus a sufficient portion of the nerve roots are removed the regeneration is always slight, eye spots do not develop and the animal never shows any recovery from its sluggish unresponsive condition, *i. e.*, it behaves in all respects like a headless animal. On the other hand, where the roots are largely intact, regeneration is more rapid and proceeds farther, eye spots often appear and the recovery of motor activity and apparent spontaneity frequently occurs to a very marked extent.

[1]Child, "Studies, etc., VI.," Fig. 43, p. 526.

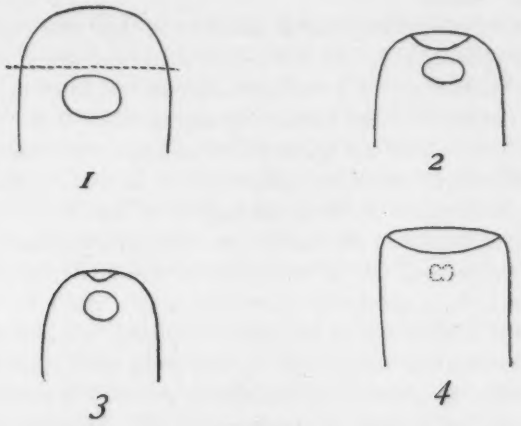
Since my later experiments confirm in all respects my earlier work, it seems unnecessary to describe them in detail and to figure all the various methods of operation and the results. I can only conclude that the apparent absence of effect of ganglionic removal upon regeneration in certain cases is due to one of two things, viz., failure to remove the ganglia completely, or the presence of the intact nerve roots. Morgan's experiments do not in any way prove that the central nervous system does not exert an influence upon the rapidity and amount, and so far as the sense organs are concerned, upon the character of regeneration in the polyclads.

As regards one point, however, Morgan's results as stated in her paper disagree so completely with my own that some further consideration is necessary. In the concluding paragraph of her paper the statement appears that "regeneration of the anterior tip of the worm, that is when the worm has been cut off anterior to the ganglia, occurs in the absence of the ganglia as well as when they are present." Individuals with the ganglia removed and the end cut off anterior to the ganglionic region regenerated as rapidly and completely as controls with uninjured ganglia and the anterior end cut off at the same level. In these experiments the ganglia were removed by using the cut end of a straw as a punch and after the wound thus made had healed the anterior region of the head was cut off.

I have performed this experiment a large number of times and on various species of Leptoplanidæ and with essentially uniform results, viz., that in all cases where the ganglia were actually completely removed, regeneration was less rapid and less complete than in control experiments with uninjured ganglia. Moreover the larger the portion of the nerve roots removed in addition to the ganglia themselves, the less rapid and less complete the regeneration. The operation is by no means easy to perform successfully and in many cases larger or smaller portions of the ganglia remain: such cases show all gradations from complete regeneration to a condition essentially like the pieces from which the ganglia are totally absent, but they must of course be regarded as unsuccessful experiments for our present purpose.

In my experiments the ganglia were removed with a straw in

the manner described above: two weeks later, after the wound had completely healed and the ganglionic region was filled in with new tissue, the anterior end of the head region was removed by a cut as indicated in Fig. 1. At the same time the anterior ends were removed at the same level from another series of individuals with uninjured ganglia.



FIGS. 1–4.

The condition of the animals without ganglia a week after the second operation is indicated in Figs. 2 and 3, while Fig. 4 shows the condition of the controls. The difference is marked and requires no comment. After two weeks more regeneration is almost or quite complete in the controls, while the animals without ganglia remain essentially as before and regeneration never proceeds further in them.

In these experiments great care was used to be certain that the ganglia were entirely removed. In various species the ganglia can be seen quite clearly from the ventral surface and examination from this side after the operation will usually show even rather small pieces of the ganglia if they remain. Morgan does not state how the total absence of the ganglia was determined in her experiments and it seems probable that in cases where the anterior end of the head regenerated as rapidly and as completely in animals supposedly without ganglia as in those with uninjured

ganglia some portions of the ganglia remained. It is possible that in some cases where the nerve roots were largely intact regeneration might be almost as rapid and complete as when the ganglia are present, but it is certainly impossible to make an extensive series of operations which are uniform in this respect. Morgan's experiments of this kind included however, only "several" worms.

The only conclusion possible seems to be then that the central nervous system, *i. e.*, the nerve roots near their origin from the ganglia, as well as the ganglia themselves, do affect in marked degree the rapidity and amount of regeneration of the anterior regions and, at least as regards the sense organs, its character as well. Moreover, where the ganglia, or the ganglia together with the nerve roots, are removed the method of operation makes no essential difference in the result. As most experiments, not only on the turbellaria but on other forms, indicate, it is probable that the early stages of the formation of new tissue are largely or wholly independent of the nervous system, but it is difficult to understand how the nervous system of an adult animal could fail to affect the amount and rapidity of growth in a regenerating part composed largely of muscles and sense organs. Absence of such an affect would be in direct opposition to the well established fact of the functional influence of the nervous system on various parts of the organism. The rate of metabolism and consequently the rate of growth—*i. e.*, provided nutritive material is present—in such parts must be in greater or less degree dependent upon nerve stimuli. Such an influence of the nervous system upon growth must, however, be sharply distinguished from the determination of differentiation of parts: the effect of the functional stimulus in the stricter sense is primarily quantitative rather than qualitative, so far as structure is concerned. These points were emphasized in my earlier papers.

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THE FORMATION OF GERM LAYERS IN ACTINIA BERMUDENSIS VERR.

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While at the Bermuda Biological Station during the summer of 1909 I attempted to secure material for a study of the development of *Actinia bermudensis* Verrill, a viviparous actinian that is abundant between tide marks in the shaded limestone caverns along the shores of the smaller islands. Since this material proved to be lacking in the earliest stages in the development, so that the complete embryology could not be worked out, I have thought it advisable to publish the following account as it covers one of the most unsettled points in the development of anthozoans.

According to McMurrich (1891), the only well authenticated cases among the Cœlenterata in which the endoderm arises by invagination are those of *Nauthisoe* and *Pelagia*, both Scyphomedusæ. Jourdan's (1878) account of the formation of the endoderm in *Actinia aquina* he discredits on the basis of his own observations on *Metridium* (*marginatum*) *dianthus*. Kowalevskis's (1873) account of the process in *Adamsia rondeletti*, which in the original was inaccessible alike to Professor McMurrich and myself, he dismisses in the same manner saying that it was probably an error in interpretation.

Appellöf (1900) describes the endoderm formation in two species of actinians: *Urticina* (*Tealia*) *crassicornis* and *Actinia aquina*. His observations on the latter species confirm the opinion of McMurrich, namely, that there was no true invagination. In the case of *Urticina*, on the other hand, he describes and figures a true invagination, which from his figures could not be considered as an error of interpretation, since he worked with serial sections.

Faurot (1907) has described a true although rather unusual type of invagination in the development of *Sagartia parasitica* and *Adamsia palliata*.

In *Metridium*, according to McMurrich, the result of segmentation is the formation of a hollow blastula with a considerable cavity. Later the inner ends of the cells are constricted off—by the appearance of vacuoles in the line where the separation is to occur—to form the endoderm. At the time when this process is finished there appears at one pole of the blastula a slight de-

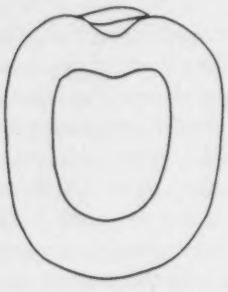

FIG. 1. Pseudogastrulation in *Metridium*. After McMurrich.

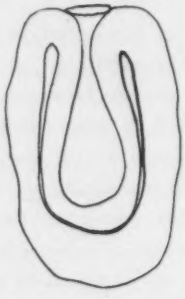

FIG. 2. Later stage in the pseudogastrulation of *Metridium*. After McMurrich.

pression which gives the embryo, when it is seen in optical section, an appearance similar to the early stages of a true invagination (Fig. 1).

When the mouth has broken through, the resemblance to an invaginate gastrula is even more complete, so that until such embryos had been seen in sections it would be almost certain to mislead any observer. In reality, however, the two layered condition had been reached before the mouth was formed. This so called "Pseudo-gastrula" McMurrich held to be the true condition in those forms in which invagination had been reported to occur.

All the material of *A. bermudensis* was obtained by slitting the adult individuals longitudinally, and then washing the embryos into a dish of sea water with the stream from a pipette. All stages including the young in which the second series of tentacles was complete, and which were ready to be liberated from the body of the parent were capable of swimming about actively by means of their cilia.

The earliest segmentation stages were never found among the

material obtained by washing out the adults, nor did sections of the mesenteries of the adult show any segmenting eggs, so the processes leading up to the formation of the blastula cannot be considered. In the section represented in Fig. 3, the blastula

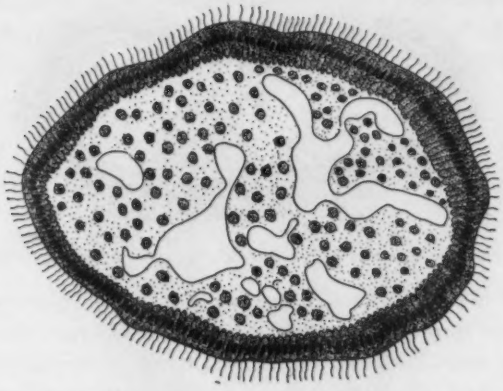

FIG. 3. Early blastula of *A. bermudensis*. Leitz 1/12 and no. 2 (reduced).

is completely formed. The cells are very numerous and of practically the same height over the entire circumference of the blastula. The interior of the blastula is to a great extent filled with a comparatively thin, lightly staining, plasma-like material, in which there are many yolk granules.

The manner in which the separation of the yolk spheres took place during the segmentation can be conjectured only, but, from the appearance of the stage just described it would seem probable that, just as in *Urticina*, there is never an extensive blastula cavity. Instead the yolk material is probably separated from the cytoplasmic portion of the cells in an early stage of segmentation.

In a later stage (Fig. 4) the blastula has become more elongated. The cells have become relatively thinner, and higher, while at one pole there is the first indication of the depression that marks the beginning of the infolding of one portion of the blastula wall to form the endoderm.

Within the interior of the blastula, there has been a marked increase in the relative amount of space unoccupied by nutritive

materials. The plasma-like substance has disappeared for a considerable proportion and the yolk spheres have undergone an apparent disintegration. Their outlines are no longer distinct, as in the earlier stages, and in many instances, they can be ob-

FIG. 4. Blastula of *A. bermudensis* just at the beginning of the invagination.

served breaking up into rather coarse granules which are being spread out through the surrounding plasma-like material.

In the stage shown in Fig. 5 the process of invagination has gone on until the section shows a well-marked early gastrula, and puts beyond any question the type of endoderm formation in this species. The character of the cells in the invaginating

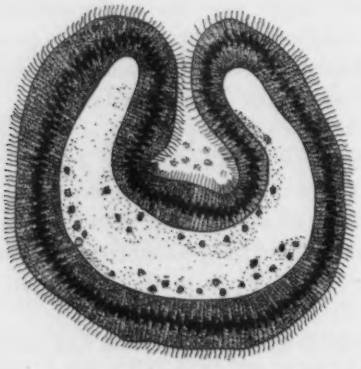

FIG. 5. Invagination of *A. bermudensis*.

area has not as yet undergone any change to distinguish them structurally from those making up the ectoderm.

The nutritive material within the original blastocœl shows practically the same conditions as noted for the stage last de-

scribed. Within the gastrula cavity there have appeared some few masses that are apparently composed of the rather coarse granules that come from the breaking up of the yolk spheres.

In the older gastrula, Fig. 6, almost all of the nutritive material has come to lie within the gastrocœl, only a comparatively few of the masses of granules, resulting from the disintegration of the yolk spheres, remaining in the original blastula cavity.

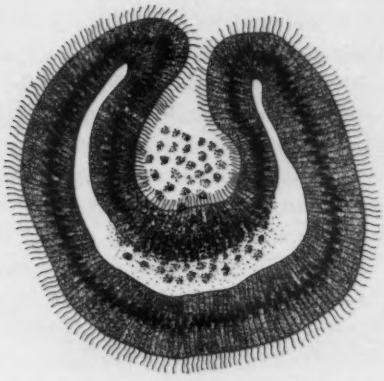

FIG. 6. Later invagination stage. *A. bermudensis.*

Appellöf (1900) describes the same sort of migration of the nutritive material during the invagination of *Urticina*. He raises the question as to whether the yolk material seen in the gastrocœl after the invagination is complete is the same as that seen in the blastocœl before invagination, and if so how the transfer could take place. Of the two possibilities in the way of a transfer he mentions: first that the yolk spheres might be absorbed by the cells of the invaginating layer and then be set free to collect in the gastrocœl; and secondly that the yolk elements pass ("sich drangen oder gedrängt werden") between the cells of the invaginating layer. According to his interpretation, his material supports the last mentioned view as many of the cells appear shrunken in diameter while yolk spheres may be seen between such cells separating their lateral walls one from another. He mentions besides that the walls at the inner ends of the cells are often very indistinct during the time when this process is

going on, although over the remainder of the gastrula wall there is no appreciable change in the characteristics of the cells.

Appellöf makes no direct mention, although his Fig. 13, Pl. 2, shows, that while the transfer of the nutritive material is taking place the yolk spheres are breaking up rapidly, so that to infer that the spheres pass through the invagination layer in their original condition is unnecessary. In the older gastrulæ of *A. bermudensis* a complete yolk sphere was scarcely ever found in the gastrocœl. While many of the masses of nutritive material still retained their identity and practically their original volume it could be observed in every instance, that the sharp outline was no longer apparent, and usually the granules were separated from one another. It is also noticeable in the section shown in Fig. 6, that nearly all of the yolk material present in the blastocœl is massed about the invaginating cell layer and that the inner ends of these cells are much less sharply defined than they were in the younger stages, Fig. 5, or than they are more laterally in the invaginating area. The central part of the invaginating area is more densely filled with granules than are those parts farther to the sides. The granules in this denser area are also markedly larger than the granules in the cytoplasm of the more laterally placed cells.

It seems, then, beyond question that in *A. bermudensis*, just as in *Urticina crassicornis*, there is an actual passing of the yolk material, in a practically unaltered state, through the layer of invaginating cells to the forming gastrocœl. As the cells of the invaginating layer approach those of the outer gastrula wall all of the yolk passes through so that the two layers come into contact and the supporting layer is secreted between them.

In an older embryo, Fig. 7, in which the formation of the stomodeum has begun, the gastrocœl—gastro-vascular cavity—still contains a considerable amount of the yolk material which now appears as distinct granules. In nearly all instances, however, the granules are arranged in groups which show clearly their origin from an originally more circumscribed mass.

In this last-mentioned stage the cells making up the endoderm have undergone a considerable change so that their histological characteristics are very different from those of the ectoderm cells.

which have retained very nearly the appearance of the original blastula cells. The endoderm cells have now become much broader in proportion to their height, their cytoplasm is much less dense than formerly, and takes the stains less readily, while the nuclei have become markedly less conspicuous. In some regions, where the body of the embryo is most contracted, especially about the region of the forming stomodeum, the outlines of the endoderm cells are very indistinct, and indeed impossible to make out at the proximal ends of the cells.

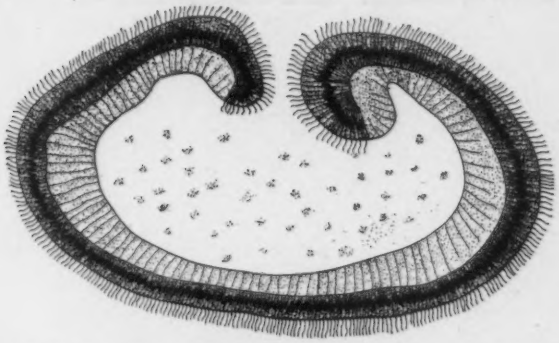

FIG. 7. Young embryo in which the stomodeum is forming. Leitz 7 and 2.

In regard to the interpretation of the optical sections shown in McMurrich's Fig. 10, Pl. XIII., it is interesting to compare my Fig. 6, with the figures of the embryos of *Actinia aquina* given by Appellöf. In the last mentioned form, where the endoderm arises by delamination, there occurs at the time of the breaking through of the mouth opening a decided thinning of both germ layers at the point where the mouth opening will appear. When the mouth has been formed the tissues are very thin all around it, and in a longitudinal section there appears about the mouth this thin area instead of the more thickened area which is found about the gastropore of an invaginate gastrula. So that in some instances at least, McMurrich's criticism of the interpretation of optical sections of whole mounts would not apply.

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